MODIFICATIONS OF RESPONSES TO ADRENERGIC DRUGS IN ARTERIAL STRIPS BY TREATMENT IN VIVO WITH EPHEDRINE AND RESERPINE

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Abstract—The aim of the present experiment was to investigate effects of ephedrine and reserpine, administered in vivo, on responses of dog isolated arterial strips to adrenergic drugs, and to study a possible mechanism involved in the reversal of blood pressure responses to dopamine. Dose-dependent contractile responses to adrenaline (A), dopamine (DA) and ephedrine (ED) were depressed in the femoral strips isolated from the ED-treated dogs as compared with those isolated from the untreated dogs. Those to noradrenaline (NA) were potentiated in low concentration and inhibited in high concentration, though those to tyramine (TY) were not altered. Relaxing and contractile responses to isoprenaline (IP) were inhibited. DA did not induce a relaxing effect but a contractile one even in the strips brought to a state of moderate tone with ED or phelypressin. In the strips isolated from the reserpine-treated dogs, contractile responses were to some extent potentiated by NA, A and DA, and significantly by ED, while those to TY were inhibited. Relaxing responses to IP were reduced and contractile responses potentiated. In the strips extirpated from the reserpine and ED-treated dogs, contractile responses to NA and A were potentiated in low concentration and inhibited in high concentration. Those to TY were inhibited in low concentration and tended to be potentiated in high concentration whereas those to DA and ED were not affected. Dose-dependent relaxing effects of DA in the dog renal and mesenteric strips contracted previously by KCl after phenoxybenzamine were attenuated by treatment with ephedrine in vivo. The results suggested that the dopamine reversal in the blood pressure may be mainly due to actions other than its peripheral effect on the blood vessels.

Adrenergic drugs and their mode of action have been generally classified as acting directly on the receptors, releasing noradrenaline from sites of the adrenergic nerve terminal and those with a mixed action, principally on the basis of modified adrenergic responses in animals treated previously with chronic denervation, cocaine or reserpine (1). In particular, the results in the reserpine-pretreated animals fully confirm the concept of direct and indirect effect, the latter being abolished by depletion of noradrenaline stores.

Concerning dopamine, the modifications of responses to dopamine by denervation or cocaine led to classification of dopamine as a directly-acting amine (2, 3, 4). Studies have also been done on the effects of dopamine after pretreatment with reserpine, and because the pretreatment reduced but not eliminated pressor response to dopamine, it was proposed that dopamine has a partial indirect action (5, 6). The authors later reported

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that pressor response to dopamine was eliminated and reversed to depressor response (dopamine reversal) by repeated administrations of ephedrine (7) or by combined use of reserpine and ephedrine (8), while pressor response to noradrenaline or adrenaline was potentiated.

The present experiments were undertaken to investigate influences of ephedrine and reserpine administered in vivo on responses to adrenergic drugs including dopamine using the isolated arterial strips, and to determine whether or not those events observed in the blood pressure were apparent in the vascular level.

MATERIALS AND METHODS

Mongrel dogs weighing 8 to 14 kg of either sex were used, and were anesthetized with i.p. injections of pentobarbital sodium 35 mg/kg in the control untreated animal and 20 mg/kg in the reserpinized animals.

The superior mesenteric, renal and femoral arteries were isolated from dogs after bleeding. The isolated vessels were cut spirally into strips and these strips (2 cm long and 2 mm wide in the superior mesenteric and renal arteries, and 2 cm long and 3 mm wide in the femoral arteries) were suspended in a 20 ml muscle chamber containing Krebs-bicarbonate solution maintained at 37°C. A gas mixture of 95% O₂-5%CO₂ was continuously bubbled through the muscle chamber. The strips were attached to isometric force transducers (Nihonkohden Kogyo Co., Tokyo, Japan), and the tension was recorded on a polygraph (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension of the small artery such as the superior mesenteric and renal artery was adjusted to 1 g while 4 g was applied to the femoral artery. The femoral strips had been allowed to relax and equilibrate for 180 min, and other strips for 120 min before the experiment was started. The Krebs-bicarbonate solution used in the experiment contained 117.7 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24.4 mM NaHCO₃ and 10 mM dextrose. This solution also included 0.24 mM of sodium bisulfite in order to inhibit oxidation of catecholamines.

The following drugs were used; 1-noradrenaline bitartrate monohydrate (Sigma Chemicals), 1-adrenaline bitartrate (Sigma Chemicals), dopamine hydrochloride (Nutritional Biochemicals), dl-isoprenaline hydrochloride (Sterling Research Institute) and tyramine hydrochloride (Tokyo Kasei Kogyo) were dissolved in 0.01 N-HCl solution. dl-ephe- drine hydrochloride (Dainippon Pharmaceutical) and phelypressin (Octapressin, Sandoz Pharmaceutical) were dissolved in distilled water. These stock solutions were kept frozen prior to use and used within one week. Working solutions of desired concentration for experimental use were made by diluting the stock solution with Krebs-bicarbonate solution and were freshly prepared before each experiment. Commercially available reserpine (Serpasil, Ciba) and sodium pentobarbital (Nembutal, Abbott Laboratories) were also used.

In vivo, ephedrine was injected into the cannulated femoral vein, and repeated administrations were accomplished as follows at intervals of 30 min: 1 mg/kg×1 time, 3 mg/
kg × 1 time, 5 mg/kg × 1 time, 6 mg/kg × 1 time and 7 mg/kg × 5 times (total 50 mg/kg), and 30 min later, sections of the artery were isolated. Reserpine 0.5 mg/kg was administered i.m. 24 hr prior to the experiment. For combined administration of reserpine (0.5 mg/kg) and ephedrine (total 50 mg/kg), ephedrine was similarly injected i.v. 24 hr after the intramuscular treatment with reserpine.

To study quantitatively the effect of drugs in in vitro experiments, the technic of cumulative dose-response curve was employed. Individual addition of drug solution to a muscle chamber was 0.2 ml or less. In order to contract the strips previously, phelypressin \(1.25 \times 10^{-6}\) U or ephedrine \(5 \times 10^{-5}\) M was added to the chamber and after attaining the plateau, the adrenergic drug was additionally administered. For observation of the relaxing response to dopamine, the strips were exposed to phenoxybenzamine \(10^{-6}\) M for 60 min and washed during the next 5 min. And allowing 30 min for equilibration, KCl \(5 \times 10^{-3}-2.5 \times 10^{-2}\) M was added in order to contract the strips previously, and dopamine was additionally administered about 35 min later (9). All concentrations in the text refer to final concentration of free base in the muscle chamber and are expressed in terms of molarity.

The maximum contraction against basic 1 or 4 g tension was expressed as absolute tension increased, while the maximum relaxation was expressed as per cent relaxation and one hundred per cent relaxation was equivalent to tension increased previously with KCl or phelypressin.

The results for statistical analysis are expressed as mean value±standard errors of the mean and significance of the difference is investigated by use of Student's t-test (P< 0.01-0.05).

RESULTS

Responses to adrenergic drugs in the femoral strips isolated from the untreated or ephedrine-treated dogs

Adrenaline, noradrenaline, dopamine, tyramine and ephedrine elicited dose-dependent contractions in femoral strips isolated from the untreated dogs as shown in Fig. 1, and all these contractions were inhibited completely by phenoxybenzamine \(10^{-6}\) M administered 40 min previously. Isoprenaline brought about a biphasic effect, i.e., relaxation in low concentrations \((5 \times 10^{-8}-10^{-6}\) M) and contraction in high concentrations \((5 \times 10^{-6}-10^{-4}\) M) as seen in Fig. 2. The former relaxations were inhibited by propranolol \(10^{-6}\) M added 30 min previously and the latter contractions by phenoxybenzamine \(10^{-6}\) M added 40 min previously. When the strips were brought to a state of moderate tone with phelypressin \(1.25 \times 10^{-6}\) U, isoprenaline showed similar responses to those seen without phelypressin (Fig. 2).

In the femoral strips isolated from the ephedrine-treated dogs, contractile responses to adrenaline, dopamine and ephedrine were inhibited significantly as compared with those seen in the untreated strips, though those to tyramine were not altered. Those to noradrenaline were potentiated in low concentrations \((5 \times 10^{-9}-10^{-7}\) M) and inhibited in high concentrations \((5 \times 10^{-7}-10^{-5}\) M) (Fig. 1). Both relaxing and contractile responses to iso-
FIG. 1. Dose-response curves to adrenergic drugs in femoral strips isolated from the untreated or ephedrine-pretreated dogs. Vertical bars represent standard errors. Significant difference from the untreated (*; P<0.05, **; P<0.01).

FIG. 2. Dose-response curves to isoprenaline in femoral strips isolated from the untreated or ephedrine-pretreated dogs. Left panel: experiment on the strips without previous contraction. Right panel: experiment on the strips contracted previously with phelypressin 1.25x10^-1 U. Further explanation as in Fig. 1.

FIG. 3. Dose-dependent contractile responses to dopamine in the femoral strips isolated from the ephedrine-pretreated dogs and contracted previously with phelypressin or ephedrine. Vertical bars represent standard errors.
prenaline were depressed as shown in Fig. 2.

In the femoral strips which were isolated from the ephedrine-treated dogs and contracted previously with ephedrine $5 \times 10^{-5}$ M or phelypressin $1.25 \times 10^{-6}$ U, additional application of dopamine resulted in further dose-dependent contractions as seen in Fig. 3. Relieving responses to dopamine in the renal and mesenteric strips isolated from the untreated or ephedrine-treated dogs

Renal or superior mesenteric strips isolated from the untreated dogs were treated with phenoxybenzamine $10^{-5}$ M for 60 minutes, then washed and contracted previously with KCl $5 \times 10^{-3}$-$2.5 \times 10^{-2}$ M. In these strips, additional administration of dopamine induced dose-dependent relaxations. These relaxing responses to dopamine in the strips isolated from the ephedrine-treated dogs were reduced (Fig. 4). This reduction in the relaxing responses was significant in the renal strips but not in the superior mesenteric strips.

Responses to adrenergic drugs in the femoral strips isolated from the reserpine-treated dogs

In the femoral strips isolated from the reserpine-treated dogs, dose-dependent contractile responses to adrenaline, noradrenaline and dopamine tended to be potentiated as compared with those seen in the untreated strips, but the potentiations were only statistically significant at low concentrations of noradrenaline (Fig. 5). Those to tyramine were reduced and significant differences were obtained at low concentrations, whereas those to ephedrine were potentiated significantly at all concentrations (Fig. 5). Relaxing responses to isoprenaline at low concentrations tended to be decreased while contractile responses at high concentrations were increased (Fig. 6).
Responses to adrenergic drugs in the femoral strips isolated from the ephedrine and reserpine-treated dogs

The femoral strips were isolated from the dogs treated concurrently with ephedrine and reserpine. In these strips, dose-dependent contractile responses to adrenaline and noradrenaline were potentiated in low concentrations but inhibited in high concentrations, and the potentions in the responses were significant in low concentrations of noradrenaline while the inhibition was significant in high concentrations of adrenaline (Fig. 7). The responses to dopamine were not altered, whereas those to tyramine were decreased significantly in low concentrations but tended to be increased in high concentrations. Those
to ephedrine tended to be diminished. Relaxing responses to isoprenaline were to some extent reduced and contractile responses to isoprenaline were potentiated as shown in Fig. 8.

**DISCUSSION**

In the reserpine-pretreated strips, contractile responses to adrenergic agonists, such as adrenaline, noradrenaline, and dopamine which acted directly on the alpha receptors tended to be potentiated. This supersensitivity induced by reserpine has been described previously by many investigators (10–19). For this mechanism, it has been proposed that
reserpine acts at the effector level to increase either the functional or anatomical receptor area. Carrier et al. (19) reported that not only catecholamine but also potassium ion and acetylcholine exhibited supersensitivity after reserpine, and they proposed as the mechanism involved that reserpine increases the permeability of the muscle to calcium and acts in addition at some other calcium site to increase the availability of calcium for contraction. The reserpine supersensitivity to agonists was not clearly observed in the dog femoral strips, while the supersensitivity was reported to be more markedly observed in the rabbit aortic strips. This difference in the supersensitivity might be related, to some extent, to the fact that the sensitivity of rabbit aortic strips to amine is higher than that of dog femoral strips (20, 21).

Responses of the strips to ephedrine was potentiated most after reserpine. Ephedrine owes part of its peripheral action to a direct effect on receptors but also induces release of noradrenaline (22); in addition, ephedrine and reserpine, when administered concurrently, are thought to cause a facilitated release of endogenous amines (23). In addition to the mechanisms mentioned above, this facilitation in release of amines by ephedrine and reserpine may be partly involved in the ephedrine supersensitivity after reserpine.

In the ephedrine-treated strips, contractile responses to noradrenaline, adrenaline, dopamine, ephedrine and isoprenaline were depressed. Patil et al. (24) reported that the contractile responses to phenylephrine and histamine in the rabbit aorta were equally reduced after pre-exposure to ephedrine, and considered that selective blockade of the receptors and non-selective depression of the effector organ might be involved.

Another possible explanation is that ephedrine acts as an antagonist to noradrenaline (25). The present authors proposed that repeated injections of ephedrine depressed the membrane permeability to calcium and reduced intracellular calcium available to the contractile apparatus since the inhibition in responses to adrenaline and noradrenaline were recovered by using the high calcium bathing solution (26).

Contractile response to noradrenaline was especially potentiated at low concentrations. Dose-response curves to noradrenaline in guinea-pig vas deferens were also shifted to the left of the control curves during continuous exposure to ephedrine derivative, amphetamine (27). As to tissue uptake of catecholamine, it is well documented that the uptake is greater with noradrenaline than with adrenaline (28). Therefore, the potentiation in the responses to low doses of noradrenaline is thought to be based on an inhibition in tissue uptake of exogenous noradrenaline by ephedrine and reserpine (28-30).

After combined treatment with reserpine and ephedrine, contractile responses to adrenaline, noradrenaline, dopamine, ephedrine and isoprenaline were not modified in a specific manner and exhibited intermediated modifications among those after respective treatment with ephedrine and reserpine.

Concerning tyramine, as indicated in the review by Trendelenburg (14) there is extensive evidence that the primary mode of action of tyramine is indirect, i.e., via the release of endogenous noradrenaline, while many other investigators propose that action of tyramine is predominantly a direct one on the alpha adrenergic receptors with relatively little release
of endogenous amine (31-38). Dose-dependent contractile responses to tyramine were significantly reduced at low concentrations in the reserpine-treated and reserpine-ephedrine-treated strips, the result implying that tyramine has an indirect action. In the meantime, the residual contractile responses to tyramine are thought to be the result of a direct effect of tyramine. Thus, it appears that tyramine has both indirect and direct action on the adrenergic receptors.

The alterations in the responses of strips to ephedrine after reserpine or ephedrine were, in general, similar to those observed with noradrenaline and adrenaline, and different from those seen with tyramine. Ephedrine is therefore thought to act mainly on the adrenergic receptors. It was reported that the mechanism by which amphetamine, ephedrine derivative and tyramine release noradrenaline is different and possibly these substances are not acting on the same pool of noradrenaline (39). Therefore, the difference between tyramine and ephedrine in the alteration after reserpine or ephedrine may be the result of differences in the direct action and in the mechanism by which both amines release endogenous noradrenaline.

As regards dopamine, contractile responses tended to be potentiated in the reserpine-treated strips and inhibited in the ephedrine-treated strips, these alterations in the responses being generally similar to those seen with adrenaline and noradrenaline, and being rather different from those observed with tyramine. The reduction in the contractile responses to dopamine after ephedrine may be not due to the depletion of endogenous amine by ephedrine, since contractile responses to directly-acting adrenergic agonists are similarly depressed. These results imply that dopamine acts directly on the adrenergic receptors.

Pressor effect of dopamine on blood pressure is not eliminated after reserpine, but eliminated and reversed to a depressor one after ephedrine or a combined use of reserpine and ephedrine. In the femoral strips, contractile responses to dopamine were markedly inhibited after ephedrine, though the responses tended to be potentiated after reserpine. These results offer a plausible explanation for dopamine reversal in blood pressure induced after ephedrine. However, responses to other adrenergic agonists, such as adrenaline and noradrenaline, were similarly depressed in the ephedrine-treated femoral strips, notwithstanding that pressor responses to adrenaline and noradrenaline were potentiated in blood pressure, after ephedrine (7). Therefore, the reduction in the contractile responses to dopamine in the strips is not a specific mechanism involved in the dopamine reversal.

It has been reported that dopamine caused a vasoconstriction in the femoral beds and a vasodilation in the renal and superior mesenteric beds in in vivo experiments on dogs (40), and that the vasoconstriction in the femoral artery was reduced while a marked vasodilation in the superior mesenteric artery still occurred after ephedrine (7). In in vitro experiments on arterial strips, dopamine did not induce relaxation but elicited further contraction in the ephedrine-treated femoral strips contracted previously with ephedrine or phelypressin, whereas dopamine induced relaxation in the renal and superior mesenteric strips pretreated with phenoxybenzamine and KCl. This relaxation was proposed to be inhibited by treatment with drugs in vitro (9, 41, 42), and was depressed after in vivo treatment with ephedrine.
This reduction in the vascular relaxation after ephedrine does not account for the dopamine reversal.

Therefore, the dopamine reversal in blood pressure may be principally due to some other action of dopamine.

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